TRANSACTIONS OF

THE ROYAL SOCIETY OF CANADA

SECTION III

CHEMICAL, MATHEMATICAL,
AND
PHYSICAL SCIENCES

THIRD SERIES—VOLUME LII—SECTION III

JUNE, 1958

OTTAWA
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SECTION III

A SYMPOSIUM ON PHYSICAL METHODS OF ORGANIC CHEMISTRY

I. The Use of Radioactive Isotopes*

LÉO MARION, F.R.S.C.

Many of the difficulties that used to face the organic chemist have been greatly reduced by the adoption of newer physical methods. It is possible because of such methods to attack and to solve problems much more readily than before, and also to tackle problems which could not previously be undertaken. Physical methods and modern concepts have had a profound influence on organic chemistry and have changed it very markedly. The main difference between modern and classical organic chemistry is that modern chemistry is three-dimensional whereas the classical is two-dimensional.

The three talks that you will hear will deal with three different physical methods. I shall try to illustrate the possibilities offered by one of these methods with the description of some results obtained by the use of radioactive tracers in the study of the biogenesis of alkaloids.

The origin of alkaloids in plants is a problem that has interested chemists working with natural products for some fifty years. Sir Robert Robinson had observed that the formula of an alkaloid could be dissected in such a way that the pieces correspond to known amino acids or substances obviously derivable from amino acids. In 1917 Robinson evolved the hypothesis that all alkaloids were synthesized in plants from amino acids. Whenever a single carbon was missing Robinson assumed that it was supplied from formaldehyde. Support for this hypothesis was claimed in the so-called pseudo-physiological syntheses which are applications of the Mannich reaction under mild conditions that could be encountered in the plant. That such conditions could conceivably exist in the plant was no proof, however, that they did, so that pseudo-physiological syntheses, although they seemed to lend support to the hypothesis, fell considerably short of constituting a proof. Although some attempts were made to feed certain amino acids to growing plants, the results were ambiguous because of the impossibility of determining whether or not the amino acid had become incorporated into the alkaloid. Consequently, the hypothesis as to the origin of alkaloids in plants was left without any experimental foundation, and it was only with the accessibility of radioactive tracers and counting techniques after the war that significant experiments became possible.

*Presidential Address.

In such experiments it is necessary first to form an idea as to which amino acid or product derivable therefrom might be involved in the biosynthesis of the alkaloid. Then with a view to the ultimate degradation of the alkaloid, one must determine which carbon atom of the amino acid should be labelled with C^{14}, and carry out its synthesis. The labelled compound is then fed to the growing plant, the alkaloid is subsequently isolated and its radioactivity measured. Finally, the alkaloid is degraded in order to ascertain that the label is in the expected position and that there has been no randomization.

The leaves of barley contain the alkaloid gramine which is present during the first 30 days of germination only. The alkaloid which is not present in the seeds reaches a maximum concentration in the leaves after 11 days of germination. Gramine is 3-dimethylaminomethyl indole and should have for its precursor tryptophane, the only indole amino acid, although it has

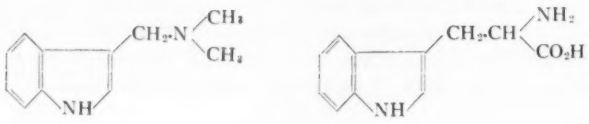

FIGURE 1

long been assumed to have a different origin. The alkaloid was originally synthesized in the laboratory by allowing a mixture of indole, formaldehyde, and dimethylamine to react, and it was this elegant synthesis that prompted the assumption that tryptophane was not the precursor of gramine.

If, however, gramine is allowed to germinate in the presence of tryptophane labelled with C^{14} in the β-position of the side-chain, the plant is found to contain radioactive gramine. When the gramine thus obtained was degraded it was established that the radioactivity was entirely localized on the CH_2 occurring between the nitrogen and the indole nucleus. This surprising result leads to the conclusion that the scission of the tryptophane side-chain does not occur at the indole nucleus, but between the CH_2 and the $CH\text{-}(NH_2)CO_2H$ grouping.

Since, however, the yield in radioactivity of the gramine was low, it was felt that this conclusion needed further proof. If tryptophane were used, labelled not only in the β-position of the side-chain as before, but also in the nucleus, then if the conclusion holds the ratio of the radioactivities of the two centres should remain unaltered in the gramine. A quantity of tryptophane labelled with C^{14} in the β-position of the side-chain was prepared and mixed with a known quantity of synthetic tryptophane labelled with C^{14} in the 2-position of the nucleus. The mixture made with accurately weighed quantities was such that the ratio of the radioactivities was $C_2/C_\beta = 0.88$. Barley, germinated and allowed to grow in the presence of this mixture for a few days, yielded radioactive gramine which was degraded according to the scheme of Figure 2.

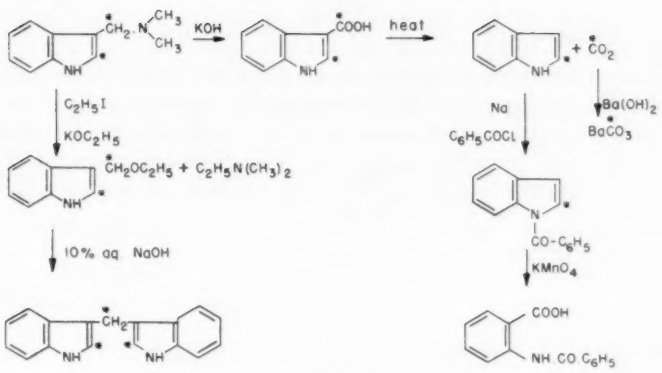

FIGURE 2

This scheme of degradation permits the isolation of the two radioactive centres individually, and thus makes it possible to re-establish the ratio of the radioactivities of the two labelled centres present in gramine. This ratio ($C_2/C_\beta = 0.88$) is the same as that in the initial tryptophane. Had the side-chain of tryptophane undergone scission at the nucleus and some of the labelled $CH_2(C_\beta)$ been used in the formation of gramine the ratio of the radioactivities C_2/C_β would certainly have been altered. The fact that it remained constant firmly establishes that no scission occurs between the CH_2 of the side-chain and the indole nucleus in tryptophane but that scission is entirely between the CH_2 and the CH of the side-chain, i.e.,

$$R—CH_2\vdots CH(NH_2)COOH.$$

The results also show that tryptophane is indeed the precursor of gramine.

Hyoscyamine is an alkaloid of the tropane group containing a substituted pyrrolidine ring. The tropane part of the molecule had been synthesized previously by Schöpf and Lehmann from succindialdehyde, methylamine, and acetonedicarboxylic acid under pseudophysiological conditions. Succindialdehyde was considered as a possible metabolite of ornithine, and indeed it has been recorded that feeding ornithine or arginine to growing *Atropa belladonna* significantly increases its content of hyoscyamine.

When adult plants of *Datura stramonium* are fed ornithine, labelled with C^{14} in the α-position, the hyoscyamine subsequently isolated from these plants is radioactive. The alkaloid was then degraded to benzoic acid as shown in Figure 3.

The benzoic acid had a specific radioactivity half that of hyoscyamine. Since both carbons 1 and 5 (on the tropane formula) produce the carboxylic group of the benzoic acid, there are two moles of acid produced by one mole of tropane and this accounts for the specific activity being halved although the total activity originally present in the base is present in the

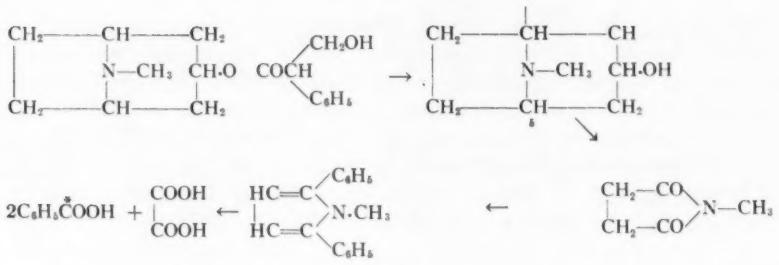

FIGURE 3

acid. Hence either carbon 1 or 5 or both are radioactive. The method of degradation used did not differentiate between these two carbons.

The Datura plant also contains a second alkaloid which is hyoscine. It has the same structure as hyoscyamine except for an added epoxide ring. In the foregoing experiment, it was found that although hyoscyamine was radioactive, hyoscine was not. Hence, either hyoscine is derived from another precursor than ornithine, or its biogenesis precedes that of hyoscyamine. It was possible to eliminate the first of these two alternatives by the use of labelled methionine. Methionine had previously been shown to be one of the precursors of methyl groups in alkaloids. It could, therefore, be expected that in *D. stramonium* grown in the presence of methionine labelled with C^{14} the methyl group of both hyoscyamine and hyoscine would be radioactive if both alkaloids were synthesized simultaneously, but that the methyl group of hyoscyamine alone would be active if hyoscine were no longer synthesized while hyoscyamine was. In fact, when the experiment was carried out, it was found that hyoscyamine was radioactive, but hyoscine was not. Hence it can be concluded that in the plant hyoscine is synthesized in the early stages of growth while in the adult plant hyoscyamine alone is actively synthesized.

The biogenesis of nicotine has been the subject of many investigations and it has been shown that the methyl group attached to nitrogen has as precursors both methionine and choline. In other plants such as barley or Ricinus, choline does not act as a precursor of methyl groups attached to nitrogen, although methionine does. When ricinus seeds are germinated in the presence of $C^{14}H_3$-betaine, the alkaloid ricinine isolated from the plant contains radioactive methyl groups. Since betaine is the oxidation product of choline, it can be concluded that in barley and in Ricinus no cholinesterase is present to oxidize choline into betaine and hence choline cannot act as a methylating agent.

It has been shown by Leete and by Byerrum that the pyrrolidine ring of nicotine originates from ornithine. It had been shown that although ornithine is the precursor of the pyrrolidine ring in hyoscyamine, putrescine, i.e., decarboxylated ornithine, is not. In the tobacco plant, however, putrescine

is a more efficient precursor of the pyrrolidine ring than ornithine. Since the ornithine used in these experiments was labelled with C^{14} only in the α-position it was expected that only position 2 or position 5 of the pyrrolidine ring would be radioactive. When the nicotine was degraded, however, it was found that the radioactivity was located in both position 2 and position 5. It is therefore probable that the transformation of ornithine to the pyrrolidine ring takes place according to the scheme of Figure 4.

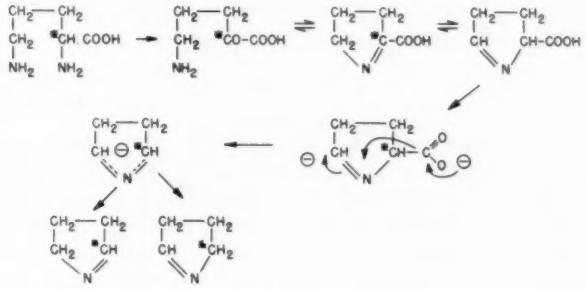

FIGURE 4

Such a scheme explains the labelling of the two positions 2 and 5 since the carbanion in acquiring a proton must produce equal quantities of the two possible aldimines. By the same method but by the use of the higher homologue of ornithine, i.e., lysine labelled with C^{14} in the α-position fed to a different species of Nicotiana, radioactive anabasine was obtained, thus showing that lysine is the precursor of the piperidine ring. In this case, however, all the radioactivity is located in the 2-position of the piperidine ring. Hence the ring closure undergone by lysine does not involve the formation of two aldimines as with ornithine, but of one only.

The biogenesis of nicotine and anabasine has thus been partially established, but the origin of the pyridine ring present in both is unknown. This six-membered heterocyclic ring was first assumed to have the same precursor as piperidine, but this has been shown not to be so. In moulds and in animals the pyridine ring of nicotinic acid has for its precursor tryptophane which is oxidized stepwise via kynurenine. In the higher plants, however, this scheme does not operate. At least, in the plants already investigated, the pyridine ring does not arise from tryptophane. One possible explanation is that the intermediate compound which must be involved between the oxidation of hydroxyanthranilic acid and nicotinic acid may be formed more rapidly by synthesis from one-carbon or two-carbon fragments than from the methabolites of tryptophane.

Stachydrine is a simple pyrrolidine alkaloid that is present in alfalfa. It is the methyl betaine of proline, and consequently should have ornithine as its precursor. When ornithine labelled with C^{14} was fed to 12-day-old

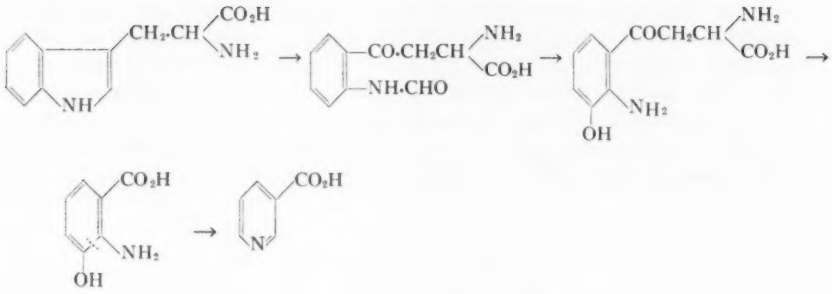

FIGURE 5

alfalfa, the stachydrine isolated from the plant was inactive and so was the proline. Since the seeds of alfalfa contain stachydrine it could be that the plant synthesizes it only at the flowering stage, just before seed formation, and this could account for the failure of the young plant to convert ornithine into stachydrine.

Since pyridoxal is a coenzyme capable of catalyzing many reactions of amino acids, a mixture of labelled ornithine and pyridoxal was fed to alfalfa, but again the stachydrine was found to be inactive, although the proline was active. Since it was known that methionine was a source of methyl groups in all the plants studied so far, it was assumed either that the plant, at that period of growth, did not contain any methionine, or that the enzyme or coenzyme required to bring about transmethylation from methionine was lacking. In order to eliminate one of these possibilities, a mixture of labelled ornithine, pyridoxal, and methionine was fed to 12-day-old alfalfa plants. The stachydrine isolated from these plants, however, was again inactive. Although proline is present in the young plant it is not converted to stachydrine, and even the addition of methionine does not bring about the conversion. It has been shown that folic acid enhances biological methylation, although it is less effective than tetrahydrofolic acid, and also that rats fed with folic acid and [C^{14}]-formate incorporate much more C^{14} into body-protein than rats deficient in folic acid. It was anticipated, therefore, that folic acid might stimulate the methylation of proline by methionine into stachydrine. Indeed, alfalfa seedlings, after administration of [C^{14}]-methionine, pyridoxal, and folic acid, yielded radioactive stachydrine (**1**).

It is possible therefore to induce alkaloid formation in a plant at a stage of growth when normally it is not synthesized. Although I have mentioned only some of the alkaloids that have been traced to amino acid precursors, there is now overwhelming evidence that, as predicted by Robinson's hypothesis, alkaloids are formed in plants from amino acids. The syntheses can take place at various stages of growth of the plant, and in some cases various alkaloids present in the same plant are synthesized at

different stages of growth. The syntheses are initiated not only by the presence of the required amino acids, but also by the presence of the proper enzyme systems.

An experimental foundation for Robinson's hypothesis of the origin of alkaloids has had to await the advent of radioactive isotopes. As shown by the examples given, the use of such isotopes allows not only the labelling of the amino acid, a step which is essential if the acid is to be recognized after it has become incorporated into the alkaloid, but also permits the location of the label in the alkaloid. The latter possibility can reveal some intermediate steps in the synthesis, as in the transformation of tryptophane into gramine (**2**).

REFERENCES

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2. For relevant references see L. Marion, Bull. soc. chim. (France), *109* (1958).

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II. Infrared Spectrometry

R. NORMAN JONES, F.R.S.C.

The recent spectacular advances in our knowledge of the physical world have come about largely through the co-operative efforts of scientists trained in different disciplines—mathematicians working together with physicists, physicists with chemists, chemists with biologists—all served by the electronics engineer who hovers in the background and hands them their tools.

In the preceding paper, our President has emphasized the part played by organic chemistry in this accelerating scientific advance. My contribution to this Symposium is to outline the place of infrared spectrometry in this scheme of things. In doing this I want at the same time to draw attention to the fact that these new physical techniques have not fundamentally altered the methods of organic chemical research, and if they are to be used to best advantage it is important that we employ them as chemists, and continue to formulate our problems in chemical terms.

In his investigation of the complex substances occurring in nature, the organic chemist has evolved a classical method based on a series of logical steps. First, the compound must be isolated in a state of purity; secondly, it must be broken down in a controlled manner to smaller pieces, each of which is simple enough to be identified by analysis and synthesis. The final phase of the problem then involves the recombination of the known fragments in the proper arrangement. Provided his reasoning was correct, and his technique adequate, the organic chemist in this way reconstitutes the original complex compound, and in doing so establishes its structure.

With the exception of X-ray diffraction, which, in favourable cases, can deal directly with molecules of great complexity, these new physical methods of investigation have not changed the basic pattern of organic chemical research. Their main service has been to facilitate the isolation of the original product, and to increase the chemist's analytical powers, enabling him to deal with larger and more complex units of structure.

We are accustomed to the idea that these new techniques of structure determination have followed rapidly on the development of new physical principles or basic advances in instrumentation. Certainly the use of isotopes, radioactive or otherwise, was not possible until the physicist had provided adequate methods for isotope fractionation and mass analysis. Likewise nuclear magnetic resonance, which will be discussed in the following paper by Professor Lemieux, is dependent on recent developments in atomic

theory and on technical advances in the production of uniform magnetic fields of high intensity.

It is therefore somewhat surprising to realize that for over half a century the organic chemist has had available adequate techniques for the measurement of infrared spectra. Furthermore all this time his library shelves have contained an extensive collection of the infrared spectra of organic compounds. From these spectra the chemist could have derived most of the important correlations between band positions and molecular structure on which the current applications of the methods are based.

Before passing on to deal with some of today's problems, we ought to take a few minutes to consider these historical matters. I suggest this for two reasons: first, because they demonstrate the importance of a proper collaborative relationship between the chemist and the physicist; secondly, because we shall have an opportunity to pay respect to an American scientist who, in this phase of his work, was much ahead of the thinking of his contemporaries.

The man to whom I refer, Dr. W. W. Coblentz, was born in Ohio in 1873. He graduated in 1900 at the Case School of Applied Science and then went to Cornell University where, at the suggestion of Professor E. F. Nicholls, he began to investigate the techniques of infrared spectroscopy (3). He soon demonstrated exceptional experimental skill and in 1903 he commenced a survey of the infrared spectra of a wide variety of organic compounds. In 1905 he published a collection of 135 of these spectra in a Carnegie Institution monograph (2). The spectra obtained by Coblentz for ortho-, meta-, and para-xylene are reproduced in Figure 1. In these curves we can discern most of the major bands detected by more modern instruments; I would draw attention particularly to the three strong bands at the right-hand end of the spectra which provide the basis for the quantitative analysis of xylene mixtures in present-day practice.

Using the Coblentz spectrometer, about 90 seconds were required to determine each point on the curve, and a complete spectrum could be mapped in 3½ to 4 hours. In contrast to this, a modern high-resolution grating spectrometer will record the complete spectrum in 3 or 4 minutes. This time difference between minutes and hours, however, is not a major consideration: we must remember that a few such measurements may serve to establish a molecular structure which might otherwise require as many years, using only the classic method of proof by chemical synthesis.

Having completed this survey of the infrared spectra of some representative organic compounds of various types, and noted some of the correlations between the molecular structure and band positions, Dr. Coblentz returned to more strictly physical fields of research, and his monograph remained unread by most organic chemists. Twenty years were to pass before the subject was reactivated by Dr. Lecomte (another physicist) in France.

It is understandable, but none the less regrettable, that in the period of

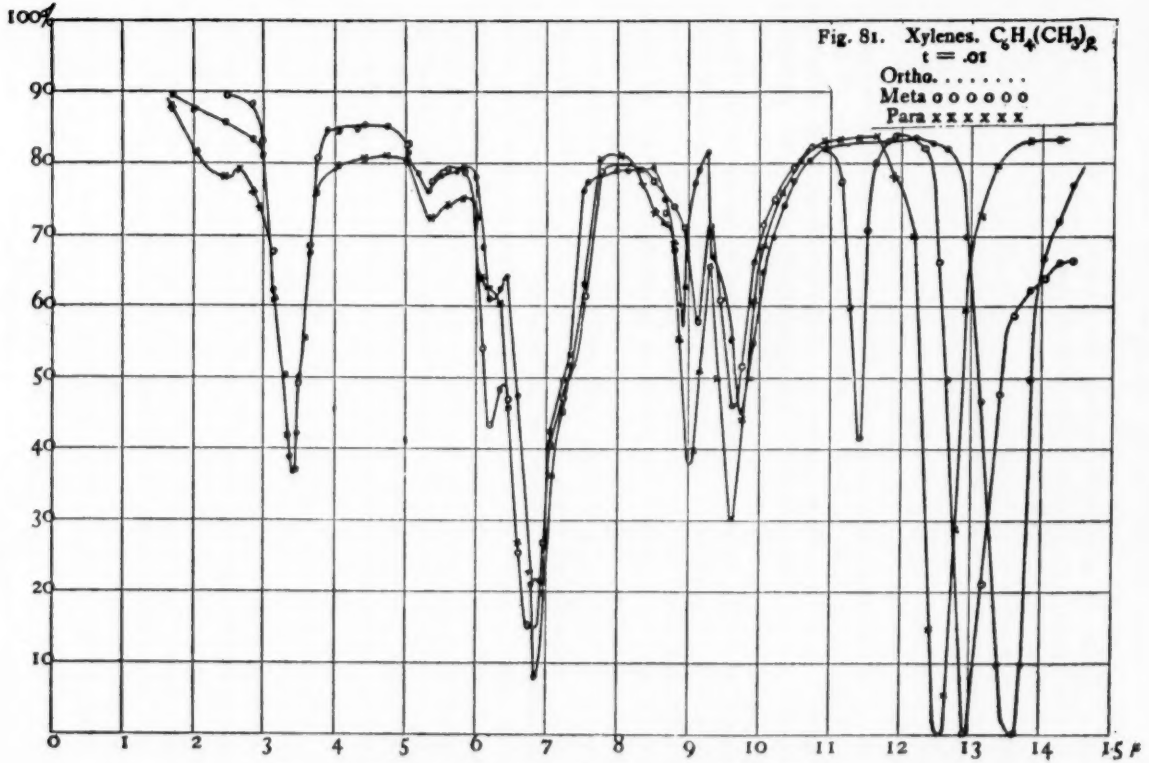

FIGURE 1.—Colbentz's spectra for xylene isomers, published in 1905.

which we speak organic chemists and experimental physicists moved in different circles and rarely consulted each others' publications. We can only speculate as to how the whole development of modern organic chemistry might have changed if the pioneer spectrographic work of this physicist had been continued by a contemporary organic chemist astute enough to recognize its implications.

In more recent times the physicist has developed a powerful and elegant calculus, based on group theory algebra, which in principle permits the infrared spectrum of a molecule to be interpreted in detail in terms of the masses of the atoms, the force constants of the bonds, and the form of the molecular vibrations. Unfortunately for the organic chemist, this technique of vibrational analysis is rigidly applicable only to molecules of high symmetry, such as methane or benzene. There is indeed much symmetry in nature, but her symmetry is of a very subtle kind, and the physicist, working within the framework of vibrational analysis by group theory, would be appalled by the interpretative problem presented by the infrared spectrum of a complex organic compound such as an alkaloid or a steroid. The chemist approaches such a problem in a different fashion. He considers the molecule, not as a single entity, but as an association of smaller structural units, benzene rings, methyl groups, double bonds and the like, each of which retains part of its individuality from one compound to another.

If we accept the physicist's concept of the vibrating molecule, we must conclude that any change in molecular structure will alter the mass distribution and symmetry, and so modify the whole infrared spectrum: all the infrared bands will be affected at least to some extent by any structural change. If, on the other hand, as organic chemists, we treat the molecule as an assemblage of quasi-independent structural units, then the infrared spectrum can be regarded as the superposition of sets of bands associated with the various structural groups. The truth, of course, lies somewhere between these two extreme abstractions, and much of the work of the chemical spectroscopist involves a judicious balancing of the two interpretations.

A detailed comparative analysis of the spectra of the symmetrical molecules amenable to theoretical analysis established quite early that the various types of vibratory motion are associated with specific regions of the spectrum. These regions are summarized in Figure 2 and it is now amply demonstrated that these generalizations are applicable also to the spectra of the more complex compounds. In Figure 2, and throughout this discussion, we shall restrict ourselves to the consideration of molecules containing only carbon hydrogen and oxygen atoms; the introduction of other atoms, such as nitrogen or sulphur, complicates the interpretation of the individual spectra, but does not modify the basic principles involved.

For purposes of discussion it is convenient to divide the spectrum into two general regions based on 1450 $cm.^{-1}$. Above this frequency the spectra of most organic compounds exhibit groups of comparatively strong bands

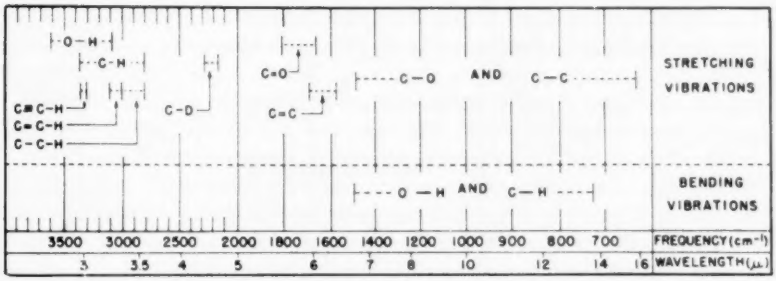

FIGURE 2.—Spectral regions associated with various types of bonds.

separated by regions of very weak absorption. The types of molecular vibrations associated with these groups of absorption bands are indicated in Figure 2. Most of these bands can be identified by the chemist as "characteristic group absorptions" associated with specific units of molecular structure. Such characteristic group absorptions can also be identified in the lower frequency part of the spectrum below 1450 cm. $^{-1}$ but, generally speaking, the absorption in this part of the spectrum is much more complex and difficult to interpret. The absorption in the 1450–650 cm.$^{-1}$ range is associated with stretching motions of C – C and C – O bonds and with bending motions of C – H and O – H bonds. Such conditions favour complex coupling interactions leading to skeletal vibrations that cannot be treated as localized in small individual structural units of the molecule. This part of the spectrum is the most sensitive to small changes in molecular structure or stereochemistry; on this account it is most useful to the chemist for the empirical identification of individual compounds and is referred to colloquially as the "fingerprint" region. The validity of this concept will be re-examined with particular reference to the spectra of steroids in a later section of this article.

An interesting, but exceptional, example of the extent to which the infrared spectrum of a complex organic compound can be treated as a combination of localized bond vibrations is provided by that of oleic acid shown in Figure 3. Some of the assignments of the lower frequency bands in this spectrum are still not fully confirmed, but organic chemists would be greatly heartened should band analyses of comparable detail ultimately be achieved for a wide variety of organic compounds.

THE C = O STRETCHING BAND

One of the molecular groups most effectively exhibiting the characteristics of a localized vibration is carbonyl. All compounds containing a carbonyl group exhibit a prominent infrared absorption band between 1900 and 1600 cm.$^{-1}$, which can be identified with the stretching vibration of the C = O bond. The location of this band within the above frequency

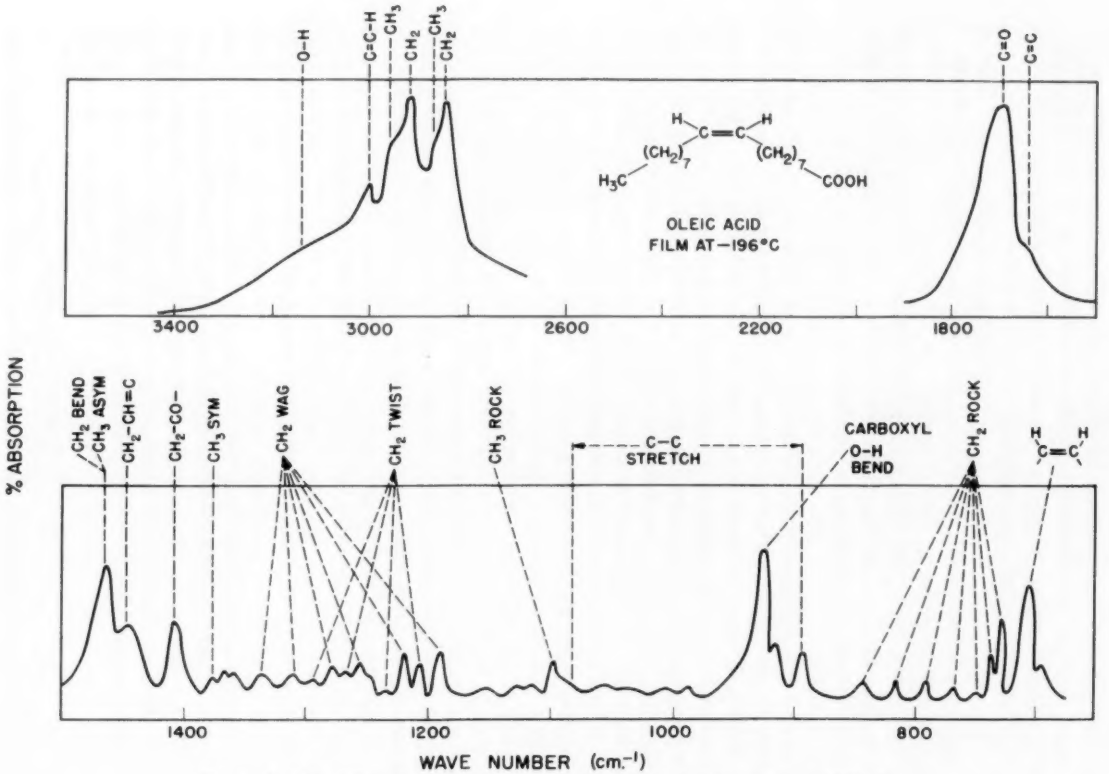

FIGURE 3.—The infrared spectrum of a crystalline film of oleic acid at —195° C.

range is determined by the molecular structure in its immediate neighbourhood, and this makes the band extremely valuable for characterizing the type of carbonyl group.

The specificity of the position of the carbonyl band maximum is well illustrated in steroids. In this very extensive family of organic compounds, C = O bonds are encountered in ketones, aldehydes, carboxylic acids, esters, and lactones. The position of the C = O stretching frequency depends not only on the type of carbonyl function, but also on its position in the steroid molecule and on the stereochemistry. From comparative surveys of the spectra of some 3,000 steroids of known structure it has been possible to classify about 150 different types of carbonyl functions in this manner (8); these band positions have subsequently proved most useful for identifying the types and positions of the carbonyl groups in the many new steroids that are continuously being synthesized or isolated from natural sources. The C = O stretching band is one of the most intense in the whole infrared spectrum, and it is possible to characterize the carbonyl functions when the total quantity of the steroid available amounts only to a few micrograms. The need to deal with such small quantities does not often arise in the course of general chemical investigations, but it is frequently a serious problem in endocrinological and other clinical investigations.

An illustration of the use of this carbonyl band specificity is provided by the two spectra shown in Figure 4. The three peaks in the curve on the left can be associated with the three carbonyl groups. The curve on the right is obtained for the compound containing an additional ketone group in the upper centre ring, and the extra band is noted near 1710 cm.$^{-1}$. The spectrographic identification of this so-called "11-ketone" group is of particular importance, since the carbonyl group at this position is sterically hindered and does not react with the chemical reagents

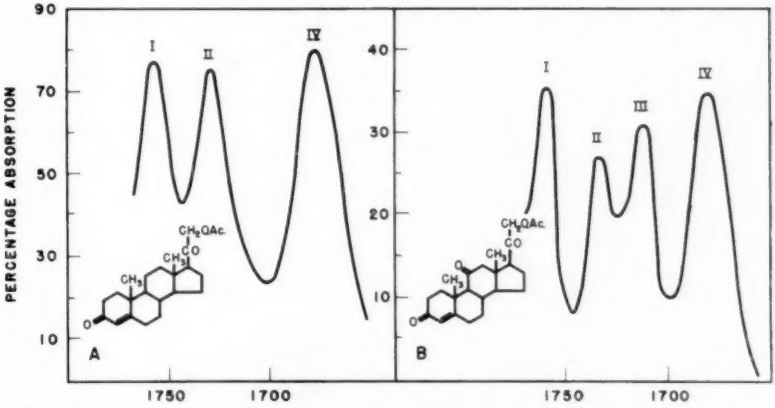

FIGURE 4.—Infrared spectra of two steroids illustrating the specificity of the C = O stretching bands. (CS_2 soln.)

commonly used for identifying carbonyl groups; on the other hand the introduction of this additional ketone group profoundly modifies the endocrinological activity of the steroid.

Small displacements in the positions of the carbonyl peaks can be helpful in sorting out equilibria among conformational isomers. Several examples

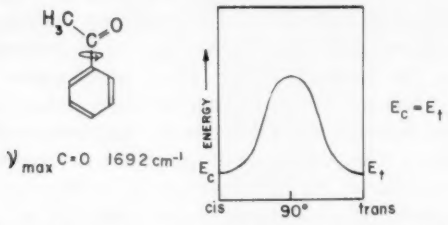

FIGURE 5.—Conformational equilibria in acetophenone.

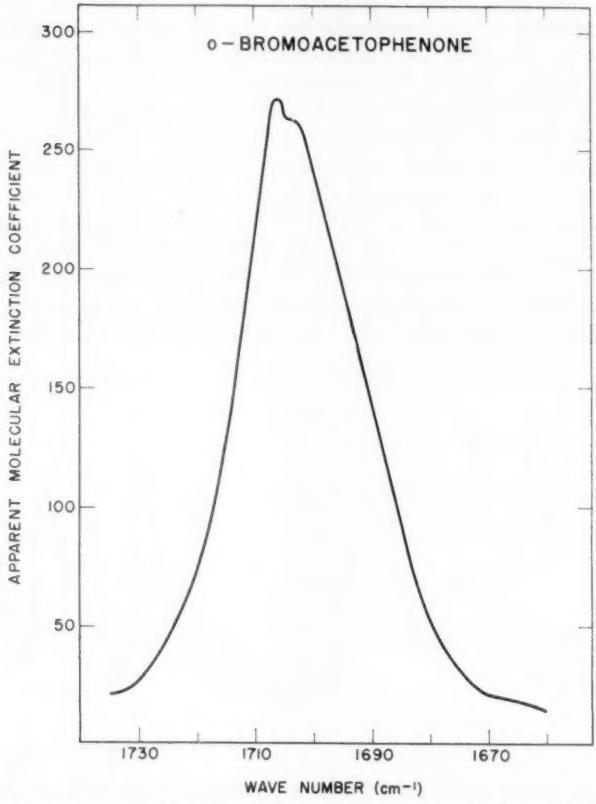

FIGURE 6.—C = O stretching absorption in ortho-bromoacetophenone (CCl_4 soln.).

of this have been encountered in studies on substituted acetophenones (**5, 6**) and an example of conformational isomerism in ortho-halogenated acetophenones is illustrated in Figures 5–8. Unsubstituted acetophenone exhibits a sharp, prominent C = O stretching band at 1691 cm.$^{-1}$ in carbon tetrachloride solution. In its most stable conformation this molecule has an approximately flat form with the C = O bond of the acetyl group lying in the plane of the benzene ring. Rotation of the acetyl group out of this plane will diminish the π-electron coupling between the carbonyl group and the ring, and raise the energy of the ground state, as indicated diagramatically in Figure 5. The spectrum of ortho-bromoacetophenone exhibits a broader C = O stretching band, with two distinctly resolved peaks (Figure 6). With increasing temperature the relative intensities of the two peaks change, the peak of higher frequency becoming stronger; this change reverses again on cooling. We are here dealing with an equilibrium between the two s-cis and s-trans conformational isomers shown in the left of Figure 7. The band at higher frequency (1706 cm.$^{-1}$) can be assigned to the s-cis conformation in which the carbonyl oxygen atom and the bromine atom are adjacent; this follows from analogy with the frequency displacements of comparable structures in which the bond relationships are fixed. From the analysis of this spectrum we can therefore conclude that in ortho-bromoacetophenone there exists some hindrance to co-planarity in both conformations but the degree of hindrance is of comparable magnitude for both forms. In the s-cis form the hindrance is largely electrostatic, resulting from the negative charges on both the bromine and the oxygen

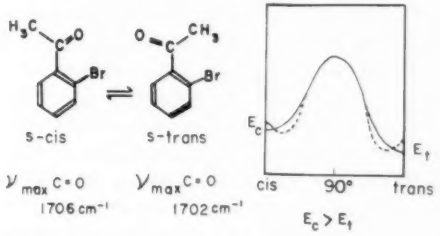

FIGURE 7.—Conformational equilibria in ortho-bromoacetophenone.

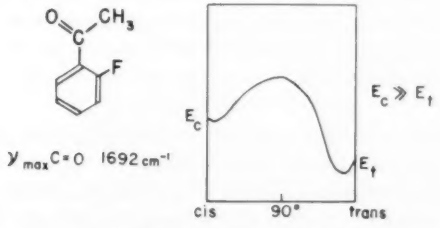

FIGURE 8.—Conformational equilibria in ortho-fluoroacetophenone.

atoms. In the s-trans conformation the hindrance is largely steric due to the van der Waals repulsion between the bromine atom and the large methyl group. This condition is expressed by the solid line in the energy diagram in Figure 7. It is more probable that for both conformations the state of minimum energy involves a small displacement from a truly planar configuration, as is indicated by the dotted line in the energy diagram of Figure 7. If the bromine atom is replaced by fluorine the spectrum shows only a sharp single peak at a lower frequency (1692 $cm.^{-1}$). The substitution of bromine by fluorine will increase the electrostatic repulsion in the s-cis conformation, but diminish the steric repulsion in the s-trans conformation. The trans structure therefore becomes so much more stable than the cis structure that the equilibrium concentration of the latter becomes negligible at room temperature (Figure 8).

Isotopic Exchange

In his presidential address, Dr. Marion has dealt with some of the applications of isotopes in organic chemistry. It is therefore appropriate that I should include some mention of the role that isotopic exchange can play in the interpretation of infrared spectra.

Radioactive isotopes, at the minute concentrations normally employed in tracer studies, have no significant influence on the infrared spectrum. The isotope affects the spectrum only by virtue of the mass change, and we therefore find the greatest effects on replacement of hydrogen by deuterium, and such exchange provides a very potent method for characterizing absorption associated with specific methyl and methylene groups. This is demonstrated by the spectrum of diethyl ketone (7) between 1500 and 1300 $cm.^{-1}$ shown in Figure 9. The spectrum of the hydrocarbon n-pentane is shown in Figure 9A. On introduction of the central carbonyl group, to form diethyl ketone, the spectrum becomes more complex, there being four main peaks, as shown by the solid line of Figure 9B. On complete deuteration all of this structure disappears (dotted curve, Figure 9B) indicating that these bands are derived from motions of C — H bonds. In the curve shown in Figure 9C, for the ketone deuterated only in the methylene groups, the bands at 1461 and 1379 $cm.^{-1}$ remain unchanged, but the pair at 1414 and 1355 $cm.^{-1}$ disappear; this establishes that the former two bands are methyl group absorptions, and the latter two methylene bands. Confirmation is provided by the spectrum in Figure 9D for the compound deuterated only in the methyl groups, which shows the opposite effect.

This deuteration technique can be equally effective in compounds of much greater complexity, such as the two steroid ketones shown in Figures 10 and 11. Cholestan-3-one (Figure 10) possesses two methylene groups vicinal to the ketone group, and the hydrogen atoms in these groups can be exchanged for deuterium by enolization (4). When this is done the

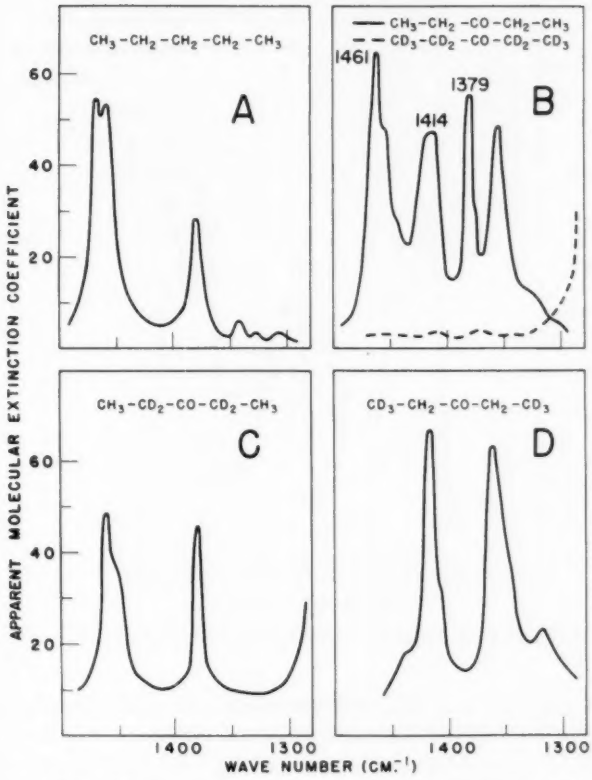

FIGURE 9.—Effect of deuterium exchange on the infrared spectrum of diethyl ketone (CCl_4 soln.).

absorption near 1419 cm.$^{-1}$ is lost indicating that the two partly resolved bands in this region are associated with these particular methylene groups. In the case of cholestan-7-one only two of the vicinal hydrogen atoms are replaced by deuterium on enolization, and these give rise to the band at 1433 cm.$^{-1}$ (Figure 11). From this latter example it will be seen that structural significance can be attached not only to the principal absorption bands, but also to quite minor bands in the spectrum.

THE FINGERPRINT REGION

There are many other interesting problems that we might discuss, but a review of this kind must of necessity be selective, and, before concluding, I should like to devote some time to the complicated problem of the interpretation of the fingerprint region of the spectrum below 1350 cm.$^{-1}$. Here, you will recall, the absorption involves both C — H and O — H bending

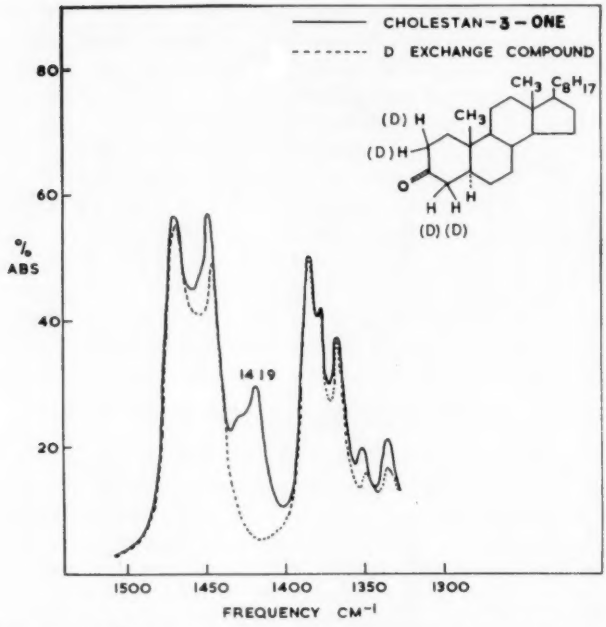

FIGURE 10.—Effect of deuterium exchange on the infrared spectrum of cholestan-3-one (CCl_4 soln.).

vibrations and C — C and C — O stretching vibrations. The large effects on the spectrum produced by small structural differences are demonstrated in the curves for the four stereoisomeric steroids shown in Figure 12. These four compounds differ only in the stereochemistry at the point of attachment of the hydroxyl group, and in the cis or trans linkage of the two rings at the left side of the molecule.

On first examination, these spectra appear to conform more closely with the physicist's concept of a vibrating molecule than with the chemist's; the complexity of the curves, and the large changes induced by the small stereochemical differences, suggest that such spectra are probably associated with general vibrations involving the whole molecular skeleton. If this were entirely true, each spectrum would be uniquely determined by the structure as a whole, and there would be little prospect of interpreting these curves in terms of more localized vibrations, or of building up a body of spectra/structure correlations from which the spectra of other steroid molecules could be predicted.

As chemists we would naturally be reluctant to accept such a negativistic hypothesis except in a last resort. The problem is a general one, not confined specifically to the steroids, but steroids do offer a particularly favourable field in which to investigate it, since several thousand of these com-

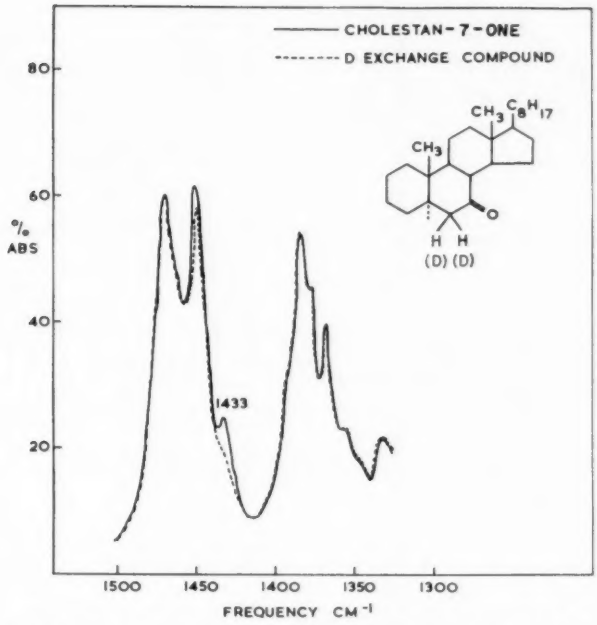

FIGURE 11.—Effect of deuterium exchange on the infrared spectrum of cholestan-7-one (CCl_4 soln.).

pounds of known structures are available for study. They all possess essentially the same alicyclic carbon skeleton, based on four condensed rings, with various substituents at known positions with known stereochemical configuration.

In a preliminary study, a band-by-band analysis was carried out on the spectra of steroids containing a common functional group. When this was done, it soon became apparent that from the multitude of bands in the spectra, certain ones could be recognized as always accompanying the presence of a particular functional group. A simple example of this is shown for the 3β-hydroxy-5β steroid structure in Figure 13. Here are shown the spectra of three compounds containing identical ring structure, but differing in the substituent at the apex of the five-membered ring. The dotted vertical lines delineate bands that are common to all three curves; these same bands are observed in the spectra of all steroids containing the hydroxyl group and steric arrangement of these compounds. In this fashion it was possible to prepare tables of bands common to particular functional groups; furthermore, provided the functional groups were well separated in the molecules, these bands could be identified, superimposed, in the spectra of steroids containing two functional groups. This is illustrated in Figure 14 where the spectrum of $\Delta^{1,4}$-androstadien-17-one is shown. The

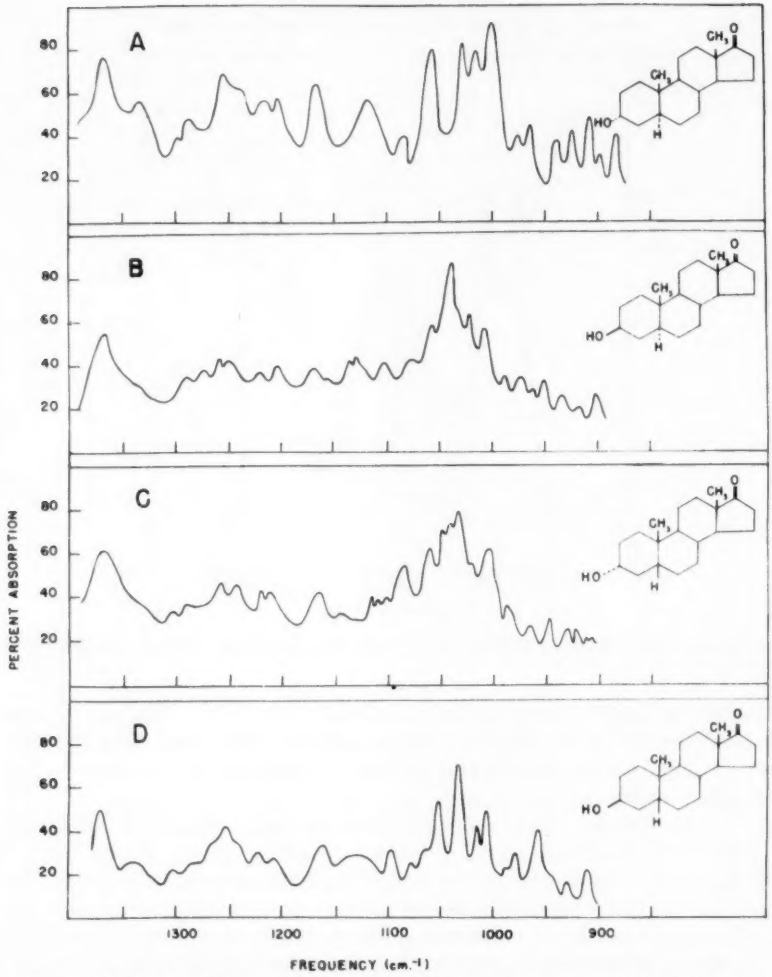

FIGURE 12.—Infrared "fingerprint" spectra of four stereoisomeric steroids (CS_2 soln.): A, 3α-Hydroxy-androstan-17-one; B, 3β-Hydroxy-androstan-17-one; C, 3α-Hydroxy-etiocholan-17-one; D, 3β-Hydroxy-etiocholan-17-one.

bands designated *A* . . . *K* can be seen in all compounds containing the di-unsaturated ketone group of the left hand ring, and the bands *a* . . . *m* in all steroids containing the "17-ketone" group at the apex of the five-membered ring. In some cases it is necessary to assign the same band to both functional groups, but at this stage of the investigation this was not considered a serious objection, since the possibility of there being superimposed bands could not be excluded.

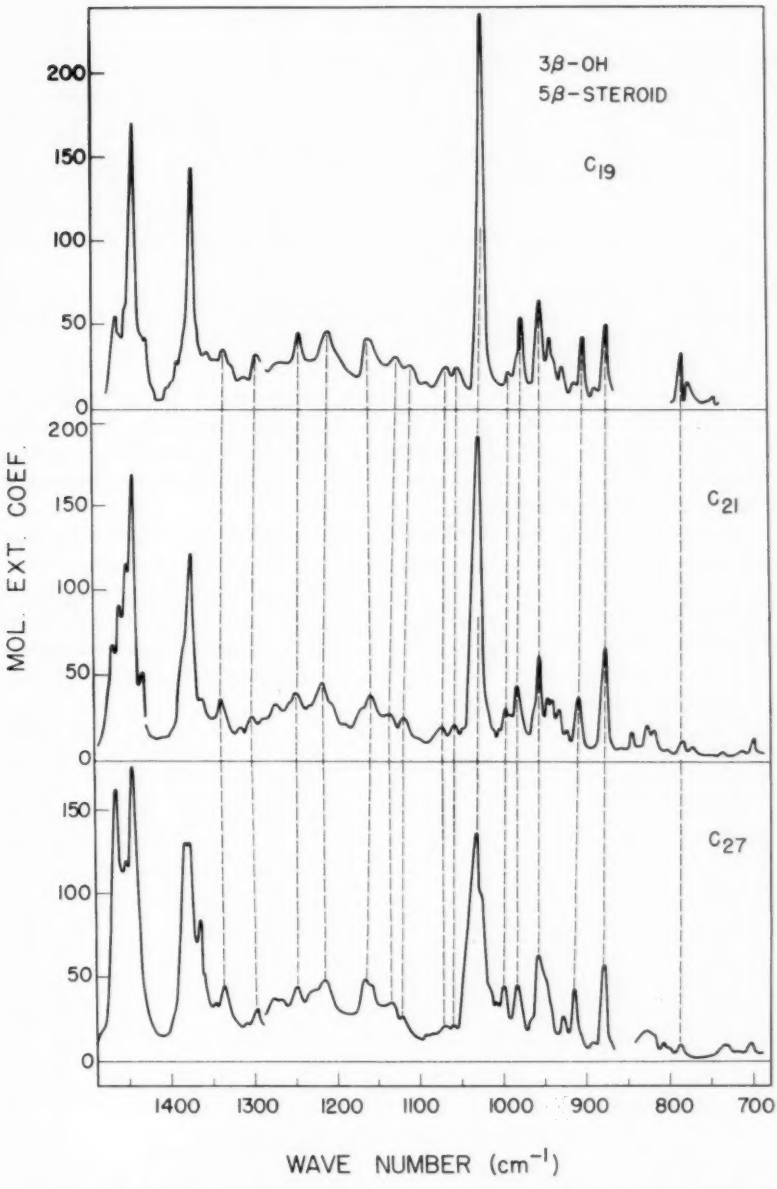

FIGURE 13.—Bands characteristic of the 3β-hydroxy-5β steroid system. Compound "C_{19}" has no substituent in the five-membered ring. "C_{21}" has an ethyl group and "C_{27}" a branched chain aliphatic substituent at the apex of the five-membered ring.

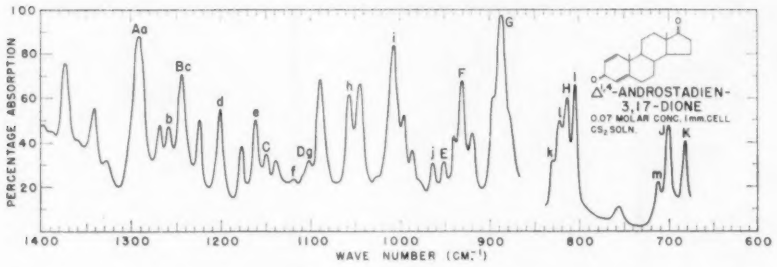

FIGURE 14.—Infrared spectrum of a di-substituted steroid. The bands *A* . . . *K* are characteristic of the di-unsubstituted six-membered ring ketone group, and the bands *a* . . . *m* of the ketonic five-membered ring system (CS_2 soln.).

Before more detailed analyses of these spectra could be undertaken, it was necessary to develop methods for obtaining spectra on an absolute intensity basis, i.e., curves plotted on an ordinate of molecular extinction coefficient (ϵ) in place of the more arbitary scale of relative percentage absorption commonly employed.

A number of technical problems had to be solved in order to do this, involving the use of a spectrometer of higher resolution than those commonly used in routine analytical work. With these improved techniques, a collection of steroid spectra on an absolute intensity basis has now been accumulated; the compounds selected for this study have been the simplest steroids, mainly saturated hydrocarbons, and compounds containing only one or two functional groups.

Using these quantitative curves we were much encouraged, and not a little surprised, by the results of computations of the type illustrated in Figure 15. Here the upper curve is the observed spectrum of 3β-hydroxy-etiocholan-17-one, one of the four stereoisomeric steroids we considered previously (cf. Figure 12D). The lower curve is a composite obtained by adding at 1 cm.$^{-1}$ intervals the values for the two corresponding mono-substituted steroids (etiocholan-3β-ol and etiocholan-17-one) and subtracting the values for the saturated hydrocarbon (etiocholane). The subtraction of the hydrocarbon spectrum is required to compensate for the fact that the summation of the alcohol and ketone spectra introduces a double contribution from the skeletal component. The close similarity between the contour of this summation curve, and the spectrum of the di-substituted steroid is clearly apparent in Figure 15 and similar agreement was obtained for the curves of the other three stereoisomers corresponding to Figures 12A–12C.

It is evident, therefore, that, at least in this particular case, the finger-print absorption of the di-substituted steroid can be treated in fair approximation as the sum of two sets of component bands each characteristic of one of the functional groups. This situation becomes a little easier to

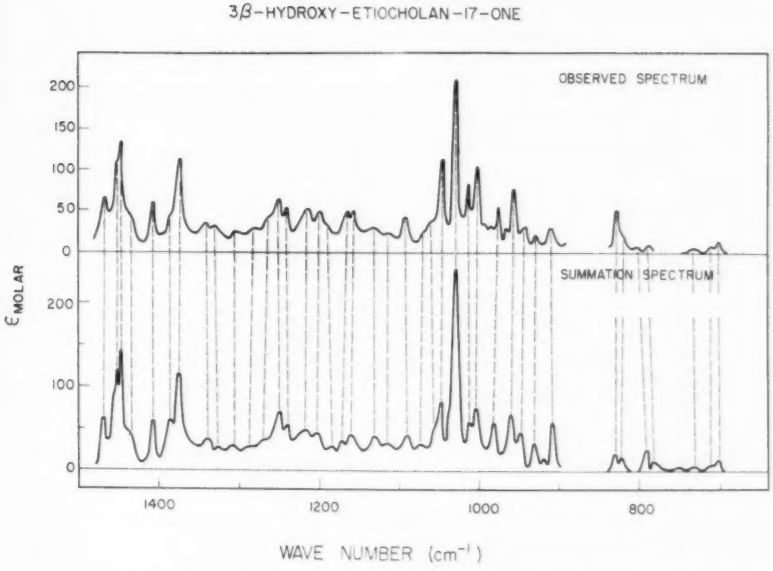

FIGURE 15.—Upper curve: Spectrum of 3β-hydroxy-etiocholan-17-one (CS_2 soln.). Lower curve. Summation at 1 $cm.^{-1}$ intervals of the spectra of etiocholan-3β-ol and etiocholan-17-one with subsequent subtraction of the spectrum of etiocholane.

understand when the three component spectra are considered separately, as shown in Figure 16, where they are all plotted on the same intensity scale. The hydrocarbon spectrum is comparatively weak, and on introduction of the oxygenated functional groups much stronger bands appear. The intensity of an infrared absorption band is determined by the change in the dipole moment during the vibration. The saturated hydrocarbons contain only C — C and C — H σ-bonds, and the charge distribution on the various atoms will be relatively small. In consequence, the dipole moment changes associated with atom displacements during the vibrations of the molecule will also, in general, be small, and the associated infrared band intensities weak. On the introduction of the oxygenated functional group, centres of greater charge displacement are introduced, not only in the C — O, O — H and C = O bonds, but also by inductive effects on the neighbouring carbon and hydrogen atoms. The introduction of the functional group therefore confers a region of potentially intense infrared absorption on the molecule. Unlike the characteristic group vibrations we cannot treat this absorption as associated with motions localized in individual bonds, but it is specific to a limited region, or zone, in the molecule, and, as such, will not be greatly modified by structural changes in the remoter parts of the molecule. It follows from this hypothesis that the presence of two

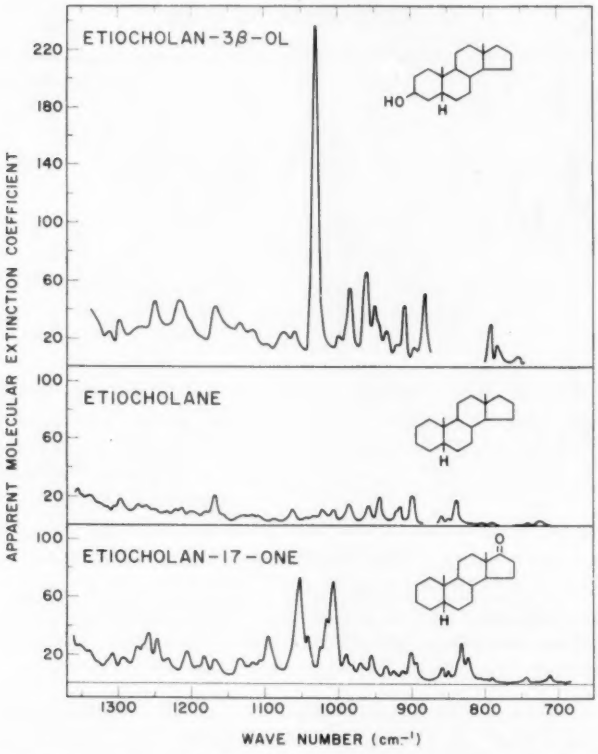

FIGURE 16.—Curves illustrating the effect of introducing oxygenated substituents on the intensities of steroid infrared spectra (CS_2 soln.).

such characteristic vibrational zones in the same molecule will confer on it a spectrum closely resembling the summation of the two zone absorption patterns.

We are now analysing a representative group of about 200 steroid spectra from this point of view and the present indications are that this type of summation analysis applies fairly generally. A simple graphical method has been developed to obtain the "zone patterns" for the various functional groups; that for the 3β-hydroxy-5β steroid is shown in Figure 17. These zone patterns can be combined in suitable pairs to simulate the spectrum of the di-substituted steroid, as in Figure 18 where the summation of the 3β-hydroxy-5β and 17-keto-5β zone patterns is illustrated. The resultant zone combination pattern is compared with the observed spectrum of 3β-hydroxy-etiocholan-17-one in Figure 19.

It would appear from the evidence at present available that this type of band analysis holds reasonably well for steroids in which the functional

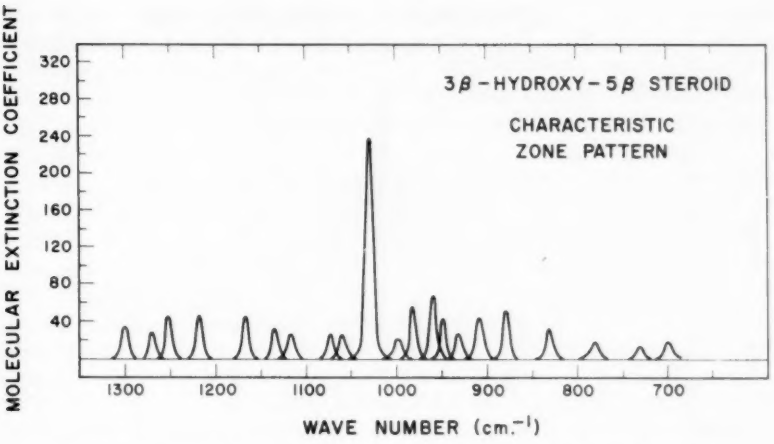

FIGURE 17.—Steroid "characteristic zone pattern" for the 3β-hydroxy-5β system.

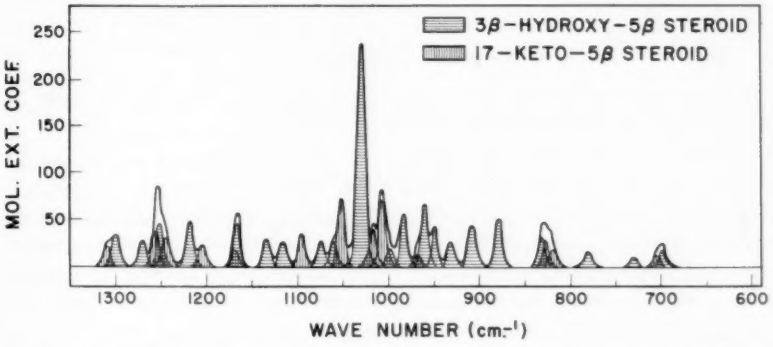

FIGURE 18.—Combination of characteristic zone patterns for two steroid functional groups.

groups are well separated. Similar behaviour would also be expected for other types of molecules built up on rigid and predominantly alicyclic structures, such as triterpenes and certain types of alkaloids. It is less likely to apply to aromatic systems where the substituent can modify the bond character at considerably greater distances through the mobile π-electron bond system. It may also be less effective in predominantly non-cyclic aliphatic molecules, or monocyclic systems where there is greater freedom for internal motions about single bonds. In such compounds the substituent will exercise a greater steric effect in determining the equilibrium conformation of the molecule, and the spectrum will be considerably altered by such conformational changes in the carbon skeleton.

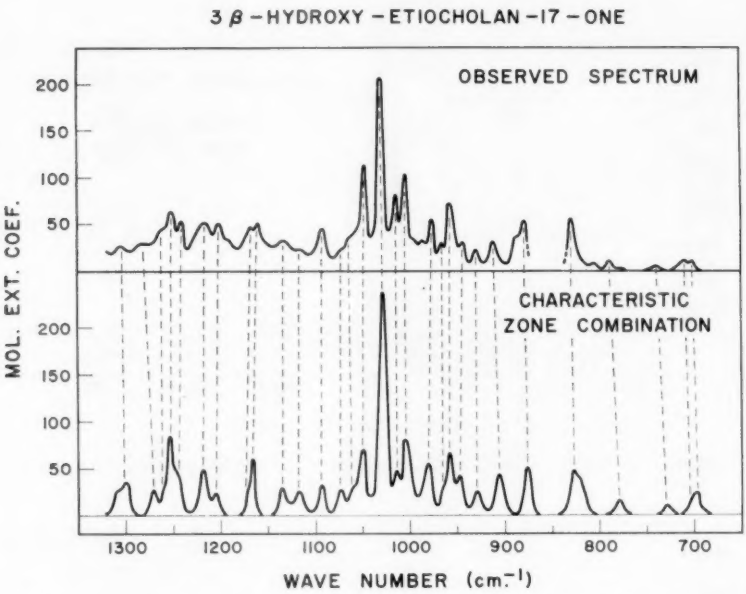

FIGURE 19.—Comparison of characteristic "zone combination pattern" from Figure 18 with the observed spectrum for a di-substituted steroid.

CONCLUSIONS

There is no doubt in my mind that infrared spectroscopy still has much to contribute to organic chemistry. Little has yet been done to investigate the effects of temperature changes on these complex spectra, and these effects might tell a good deal about conformational equilibria, and the energy barriers between different conformational forms.

High resolution grating spectrometers of commercial manufacture will shortly be making their appearance. These may not resolve much additional structure in the spectra of organic compounds measured in solution, but they will have an important consequence in permitting the ready measurement of accurate band intensities and band shapes. Increasing importance will probably be attached to the measurement of band shapes, particularly where these are asymmetrical, and measurements of this kind will be helped by electronic equipment that will permit the direct measurement of the band slope ($d\epsilon/d\nu$) and its second derivative ($d^2\epsilon/d\nu^2$).

I believe also that in the near future there will be a reawakening of interest in Raman spectrometry on the part of organic chemists, as a consequence of recent instrumental developments that greatly increase the sensitivity of this technique. Raman spectrometry and infrared spectrometry are complementary methods for studying molecular vibrations. In his structural

studies on small molecules, the physicist has relied heavily on the comparison of the two types of vibration spectra, and much of his structural analyses depends on the similarities and differences between the two. The same may prove true for these larger and more complex molecules also, and in the future the organic chemist may well utilize both techniques together.

Much of the work of the chemical spectroscopist involves a basically empirical approach to his problems. Empiricism is not very fashionable in scientific work today and much subterfuge is used to disguise it. Nevertheless there is a place for empiricism in research and this point of view has been aptly expressed in a quotation attributed in a recent biography (1) to Mr. Charles F. Kettering of the General Motors Corporation: "In research a certain amount of intelligent ignorance is essential to progress . . . for if you know too much you won't try the thing."

In conclusion I should like to acknowledge the important contributions of the several National Research Council Postdoctorate Fellows who have participated in various phases of the investigations I have discussed in this review. These include Dr. E. M. Augdahl, Dr. A. Nickon, and Dr. D. J. Whittingham in addition to those whose contributions are acknowledged more formally in the list of references cited below. In the greater part of our steroid investigations we have collaborated closely with the late Dr. K. Dobriner, Dr. T. F. Gallagher, and their colleagues at the Sloan-Kettering Institute for Cancer Research.

We are grateful to the publishers listed below for their kind permission to reproduce the figures as indicated. Carnegie Institution of Washington (Figure 1); Chemical Institute of Canada (Figures 2 and 4); Interscience Publishers Inc. (Figure 3); Canadian Journal of Chemistry (Figure 5); Kresge-Hooker Science Library Associates (Figure 9); Academic Press Inc. (Figure 12); Journal of the American Chemical Society (Figure 14).

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TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
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SECTION III

A SYMPOSIUM ON PHYSICAL METHODS OF ORGANIC CHEMISTRY

III. Nuclear Magnetic Resonance Spectroscopy

R. U. LEMIEUX, F.R.S.C.

Nuclear magnetic resonance spectroscopy involves the absorption of energy in the radio-frequency range by nuclei which are under the influence of a powerful homogeneous magnetic field. The technique can yield information on the immediate environment in molecules of atoms whose nuclei possess magnetic moments; for example, hydrogen-1, deuterium, fluorine-19, phosphorus-31, and boron-11. To date, the applications in organic chemistry have involved mainly the spectra of hydrogen-1 and fluorine-19. Only spectra of hydrogen-1 will be discussed in this communication (**1**). Furthermore, only spectra which were obtained by the author in collaboration with Drs. H. J. Bernstein, W. G. Schneider, and R. K. Kullnig will be considered. It is felt that the conclusions drawn from these spectra will suffice to illustrate clearly the rather revolutionary effect this new branch of spectroscopy is having on the practice of organic chemistry; especially with respect to the determination of such molecular properties as the arrangement of the atoms, configuration, conformation, electron distribution, and potential energy barriers.

Undoubtedly, the most important single feature of high-resolution proton magnetic resonance spectra is that their fine structures are often quite readily amenable to theoretical interpretation. Thus, a close collaboration between organic chemists and theoretical chemists is essential for maximum efficiency in the application of this new tool to the problems of organic chemistry. Fortunately, it is often possible (**2**) for the theoretical chemists to present the results of their quantum mechanical considerations in simple terms and the publication of theoretical spectra allows the organic chemist in many cases to draw his own conclusions. Of course, a great many semi-empirical correlations can be established (such as is the case in infrared spectroscopy) which are also of great value.

The proper determination of the NMR spectra of organic compounds depends on many variables such as physical state, solvent, temperature, concentration, and signal to noise ratio. A consideration of these experimental details seems extraneous on this occasion. It must be realized, however, that the proper use of a present-day high-resolution spectrometer is not a straightforward, routine operation. The instrumental problems are such that a high degree of experience is required for the operator to make reliable conclusions regarding the acceptability of spectra.

The language of NMR spectroscopy involves the four following terms of which at least a qualitative understanding is required: (1) intensity of the signal; (2) chemical shift; (3) spin–spin coupling constant; (4) half-band width. It seems desirable on this occasion to define these terms on the basis of a physical picture for the phenomenon of nuclear magnetic resonance absorption.

The spin quantum number, I, for a proton is ½. Therefore, if a proton is placed in a magnetic field, the degenerate state is split (similar to the Zeeman effect) into $2I + 1 = 2$ spin states. The energy, ΔE, which separates these two states is dependent on the strength of the applied field, H_0, the number of spin states, and the magnetic moment, μ, of the proton,

$$\Delta E = 2\mu H_0.$$

This energy difference corresponds to a frequency of transition given by the Bohr condition,

$$\Delta E = h\nu,$$

where ν is the frequency at which energy is absorbed to induce the transition. For the proton, the frequency is 40 megacycles per second for a magnetic field of about 9,400 gauss. The spectra, which will be described later on, were all measured under these conditions. Recently, a commercial spectrometer operating at about 14,100 gauss has become available. This advance in instrumentation is of great interest to organic chemists since, by nearly doubling the strength of the measured signal, it enables the use of much smaller samples. Also, the resolution is greatly improved.

Thus, having subjected protons to a magnetic field, H_0, the technique involves the detection of the absorption of energy by the protons from an alternating magnetic field set at right angles to the direction of the applied field. The alternating magnetic field is produced by passing a radio-frequency signal through a small coil around the sample which is at right angles to H_0. When the frequency of the alternating magnetic field corresponds to that required to induce the transition (that is, when there is a resonance exchange of energy) there is an efficient absorption of energy which causes a drop in the voltage of the radio-frequency signal. This drop in voltage comprises the *proton magnetic resonance signal.*

In practice, the radio-frequency signal is usually kept constant and resonance is achieved by varying the magnetic field. At 9,400 gauss, a sweep of only about one-tenth of a gauss is necessary to cover the range of resonance positions with a 40 Mc/sec radio-frequency signal for the protons of organic molecules.

As inferred above, all protons in an organic molecule do not absorb at the same position in the magnetic field. Since the intensity of the signal is dependent on the number of protons responsible for the signal, the relative intensities of the signals for different hydrogens will be the same as the relative amounts of the different hydrogens in the molecule. Thus, a

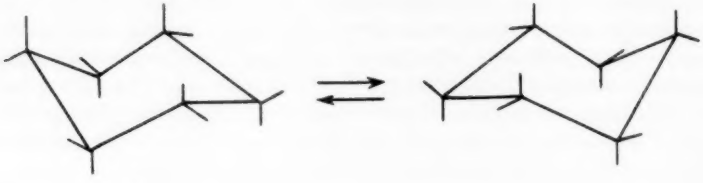
valuable application of the technique to organic compounds is to determine the relative numbers of different kinds of hydrogens in a molecule.

If all the nuclei of the hydrogens in a molecule were bare, they would obviously all produce signals at the same position in the magnetic field. However, the protons are surrounded by electrons which because of their magnetic moments provide magnetic shielding. The extent of the shielding varies with variations in the chemical nature of the hydrogen atoms since these chemical differences involve differences in electronic bonding. Thus, the nuclei of chemically different hydrogens will experience different applied magnetic fields and, consequently, at a fixed radio-frequency, resonance occurs at different applied magnetic fields. The difference between the applied magnetic fields is known as the *chemical shift*. As a rule, the greater the electronegativity of the atom to which the hydrogen is bonded the lower will be the strength of the magnetic field required to bring about the resonance with the fixed radio-frequency signal. The separations of the signals in the magnetic field are usually measured in terms of the frequency of an audio-frequency signal which is employed as a scale on the screen of an oscilloscope and these measurements most often are reported directly in cycles per second. It is to be noted, however, that the magnitudes of these chemical shifts are different for different fixed frequencies for the alternating magnetic fields. Division of these chemical shifts by the radio-frequency signal in megacycles per second yields values for chemical shifts as parts per million of the magnetic field. Expressed in this fashion the shifts are independent of changes in the radio-frequency.

The magnetic moment of a proton in a molecule interacts not only with the applied magnetic field, H_0, but also with the magnetic dipoles of other nuclei in the molecule. Fortunately, except for those between protons, the effects of such interactions usually are not observed in ordinary organic molecules which contain only carbon, oxygen, nitrogen, sulphur, halogens, and hydrogen. The interactions between the proton nuclei take place by way of an indirect coupling mechanism which involves the electrons in the molecule. Thus, a nuclear magnetic moment tends to orient the magnetic dipoles of nearby electrons which in turn orient the spins of other electrons and in turn the magnetic dipoles of other nuclei. These interactions, like the inductive effect, are rapidly attenuated with increased separation of the nuclei in the molecule and are not usually observed with the present-day spectrometer if the coupled nuclei are separated by more than three covalent bonds. The fortunate result of this fact is that the measurable fine structure for the spectrum of a proton in a molecule can usually be accounted for through a consideration of its immediate environment only. That is, it is not necessary to consider the molecule as a whole as is usually the case in infrared absorption spectroscopy.

The signal for a proton is not affected by coupling with other completely equivalent protons. This fact has a theoretical basis from symmetry considerations in the quantum mechanical treatment. Thus equivalent hydro-

gens, such as those in a methylene or a methyl group, which are separated in the molecule from different hydrogens by more than three covalent bonds all produce their signals at the same position in the magnetic field. Thus, molecules like methane, ethane, neopentane, and benzene together with groups such as methoxyl, acetyl, and quaternary methyl produce only one sharp signal. The hydrogens in certain ring compounds such as dioxane and cyclohexane can be considered to be of different kinds because of their different orientations. For example, it is established that cyclohexane (I) possesses six axial hydrogens and six equatorial hydrogens and it might therefore be expected that cyclohexane would produce two signals. However, the predominating conformations are identical and the rate of conformational change is greater than the reaction time of the machine (in the order of one-hundredth of a second), and only one sharp signal is obtained since the spectrometer is incapable of distinguishing the hydrogens. This is the case for cyclohexane. Compounds such as gamma-benzene hexachloride (II) and cis-inositol hexaacetate (III) produce only one signal for the hydrogens on the ring (3).



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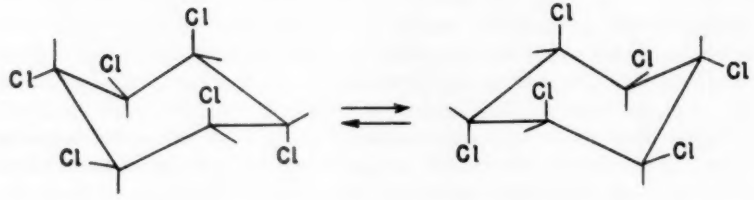

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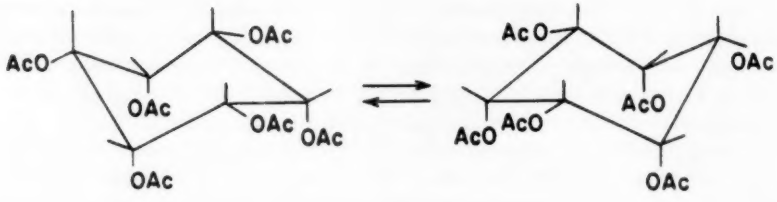


III

However, if the conformational change does not yield an identical compound, proton magnetic resonance spectroscopy can distinguish between equatorially and axially oriented hydrogens. This fact is illustrated by the spectra for the delta- and epsilon-benzene hexachlorides shown in Figure 1. The spectra clearly indicate two different kinds of hydrogens in these molecules and the intensity ratios of 1:5 (delta isomer) and 1:2 (epsilon isomer) are in agreement with the established configurations for these compounds (**4, 5**). Thus, the configurations of the isomeric parachlorals (see Figure 1) could be readily established (**3, 6**).

It was established, as inferred in Figure 1, that axial hydrogens tend to produce their signals at higher field than do similar hydrogens but in equatorial orientation. The evidence in support of this conclusion was best

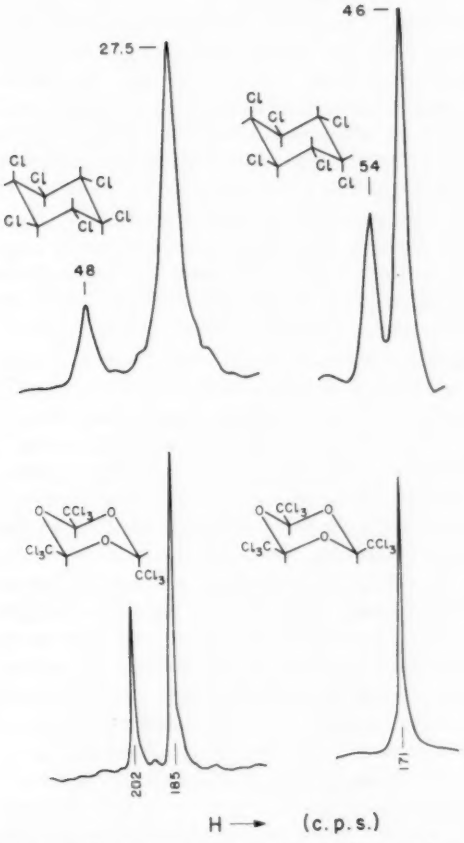


FIGURE 1.—Proton magnetic resonance spectra of δ and ε benzene hexachlorides (5) and the α- and β-parachlorals (6) in dioxane.

based (3) on a consideration (as will be seen later on) of the fine structure of signals for the 1-hydrogens of the cis and trans 4-t-butylcyclohexyl alcohols and acetates whose configurations are known with certainty. However, evidence for the conformations shown for the benzene hexachlorides in Figure 1 had previously been obtained by molecular dipole measurements (5).

If there is a chemical difference between two strongly coupled hydrogens, then the protons will not, because of their different electron shielding, experience the same magnetic field. Consequently, the protons will be at different energy levels. Since the protons are coupled, one cannot absorb energy from the radio-frequency signal independently of the other. That is, each of the protons will experience both the energy levels (spin states) of the other proton to which it is coupled. Consequently, the proton will absorb energy from the radio-frequency alternating magnetic field at two positions in the applied magnetic field and the signal for the proton will be in the form of a doublet rather than a single sharp peak as would be the case in the absence of the nuclear coupling. The peaks of the doublet are located at higher and lower field at equal distances from where the signal would be in the absence of nuclear coupling. The distance between the signals is known as the *spin–spin coupling constant*. The magnitude of the constant is a measure of the strength of the coupling between the two nuclei and has dimensions of energy. Since it is a characteristic of the molecule, it is independent of the applied magnetic field and usually is expressed in terms of the cycles per second of the audio-frequency which is used as internal standard to measure the differences in magnetic field. Lines separated by one or more c.p.s. usually can be resolved with a spectrometer operating at 40 Mc/sec.

The number of lines in the fine structure of the signal for a proton increases as indicated in Figure 2 when the chemical shift (c.p.s.) between the coupled protons is large (about four times or more) relative to the spin–spin coupling constant (c.p.s.). The situation is more complex when the chemical shift is approximately equal to the coupling constant (2).

A consideration of Figure 2 clearly indicates that, as the complexity of the coupled system increases, there may be a tendency for the signals to pile up so close to each other that the spectrometer cannot reveal all the fine structure. In fact, it is not often possible with the available spectrometers to obtain more than an envelope of signals when the coupled system contains more than four hydrogens. It is important to realize, however, that the width of an envelope at half its height (half-band width) can yield information as to the magnitude of the coupling constants involved since the greater the half-band width the stronger the coupling.

Karplus and co-workers (7) have shown that the coupling constant for two hydrogens on the same carbon is 13 c.p.s. It may be expected that a similar constant would exist for hydrogens on neighbouring hydrogens. However,

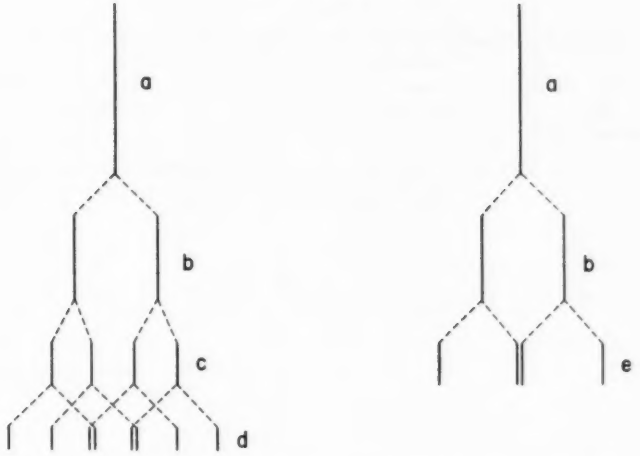


FIGURE 2.—Simple graphical approximation (first-approximation perturbation theory) of the type of NMR signals produced by an axial hydrogen which is coupled with hydrogens on neighbouring carbons: (*a*) signal if the hydrogen is not coupled with other hydrogens; (*b*) signal if the hydrogen is coupled with another axial hydrogen; (*c*) signal if the hydrogen is coupled with both an axial hydrogen and an equatorial hydrogen; (*d*) signal if the hydrogen is coupled with two axial hydrogens and one equatorial hydrogen; (*e*) signal if the hydrogen is coupled with two axial hydrogens. This procedure is useful only when the chemical shift for the coupled hydrogens is large as compared to the coupling constants.

whereas two hydrogens on the same carbon are held in substantially constant orientation relative to each other, hydrogens on neighbouring carbons may occupy a number of orientations because of the freedom of rotation about the carbon to carbon bond. Since it had been observed (**8**) in the case of ethylene compounds that trans-hydrogens are coupled 2 to 3 times more strongly than cis-hydrogens (**7**), it was reasonable to anticipate a similar situation for hydrogens on neighbouring carbons. The first clear indication that this is in fact the case was found in the proton magnetic resonance spectra of acetylated sugars (**9**). However, the assignment of the coupling constants was based (**3**) on the spectra of the isomeric 4-t-butylcyclohexyl acetates shown in Figure 3. The 1-hydrogens of these compounds are coupled with two equatorial and two axial hydrogens on the neighbouring carbons. Thus, the signal for this hydrogen in the case of the trans-isomer should involve a coupling constant for two axial hydrogens and one for an axial and an equatorial hydrogen. On the other hand, in the case of the cis-isomer, the signal should involve a coupling constant for two equatorial hydrogens as well as one for an axial and an equatorial hydrogen. Since the half-band width of the signal for the 1-hydrogen is about 3 times

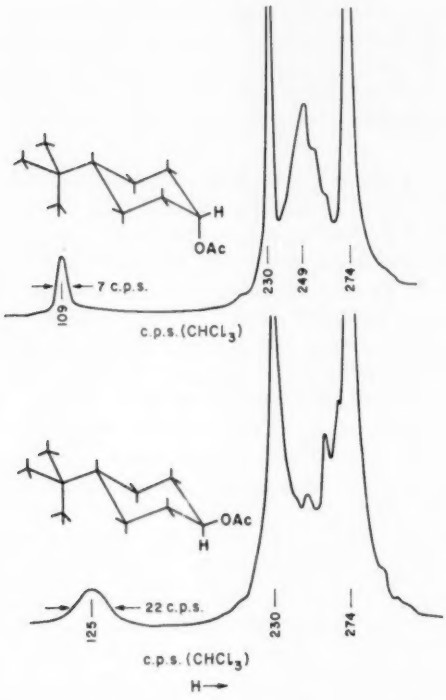


FIGURE 3.—Proton magnetic resonance spectra for the cis and trans 4-t-butylcyclohexyl acetates.

greater in the case of the trans-compound than in the case of the cis-isomer, it follows that the coupling between two axial hydrogens is substantially greater than that between two equatorial or that between one axial and one equatorial hydrogen. Since the orientation of hydrogens on the neighbouring carbons relative to each other is the same when both the hydrogens are equatorial or when one is axial and the other is equatorial, it was to be expected that the coupling would be about the same in each case. This was found to be the case through a study of the spectra of sugar acetates (see Table I) (3, 9).

Figure 4 illustrates the type of spectra which were obtained for sugar acetates (3). The four groups of signals were readily and convincingly assigned to the four different kinds of hydrogens in these compounds simply by inspection of the intensities, positions, and fine structures of these bonds. The 1-hydrogen at the anomeric centre is a unique type of hydrogen in these compounds. Being attached to a carbon which is bonded to two oxygen atoms, its nucleus should be most exposed to the magnetic field

TABLE I

PROTON MAGNETIC RESONANCE SPECTRA FOR THE ANOMERIC HYDROGEN OF ACETYLATED ALDOPYRANOSES (3)

Fully acetylated aldopyranose	Position in the magnetic field c./s.*	Spin–spin coupling constant
1. AXIAL ANOMERIC HYDROGEN		
(*a*) *Axial 2-hydrogen*		
β-D-xylose	65	6
β-D-ribose	62	5
α-L-arabinose	73	8
β-D-glucose	69	8
β-D-galactose	72	6
(*b*) *Equatorial 2-hydrogen*		
β-D-mannose	6.35	3
2. EQUATORIAL ANOMERIC HYDROGEN		
(*a*) *Axial 2-hydrogen*		
α-D-xylose	49.9	2.0
α-D-ribose	58	2.5
β-D-arabinose	46	3†
α-D-glucose	51	3.2
α-D-galactose	47	3
(*b*) *Equatorial 2-hydrogen*		
α-D-mannose	52	3

*Relative to the signal for the chloroform used as solvent and taking the mid-point between the two signals of the doublet.

†The width of the signal at half its height.

and, consequently, it should produce its signal at lower field than the other hydrogens in the molecule. Also, the anomeric hydrogen is coupled strongly only with the 2-hydrogen. Therefore, its signal should appear as a doublet. In accordance with these expectations, a doublet appears in the spectra of sugar acetates in the region 45–75 c.p.s.* Direct evidence in support of this assignment was obtained through the observation that this signal is not present in the spectra of the anomeric 1-deuterated-D-glucopyranose pentaacetate. A radio-frequency of about 6.2 Mc/sec is required to observe the nuclear magnetic resonance spectra of deuterium atoms using an applied magnetic field of 9,400 gauss. The structure of the signal for the anomeric hydrogen of sugar acetates enabled the assignment (see Table I) of conformations since the strong coupling observed for many compounds clearly indicated that the 1-hydrogen was axial and coupled with an axial 2-hydrogen. Table I also contains the positions in the field for the centre of the signals for the anomeric hydrogens. The fact that each compound with an equatorial 1-hydrogen produced the signal at lower field than did its anomer with the hydrogen in axial orientation is in agreement with what had to be expected from the spectra for the 4-t-butylcyclohexyl acetates (Figure 3) and the benzene hexachlorides (Figure 1).

*Except where otherwise noted, all chemical shifts reported herein are from the chloroform signal.

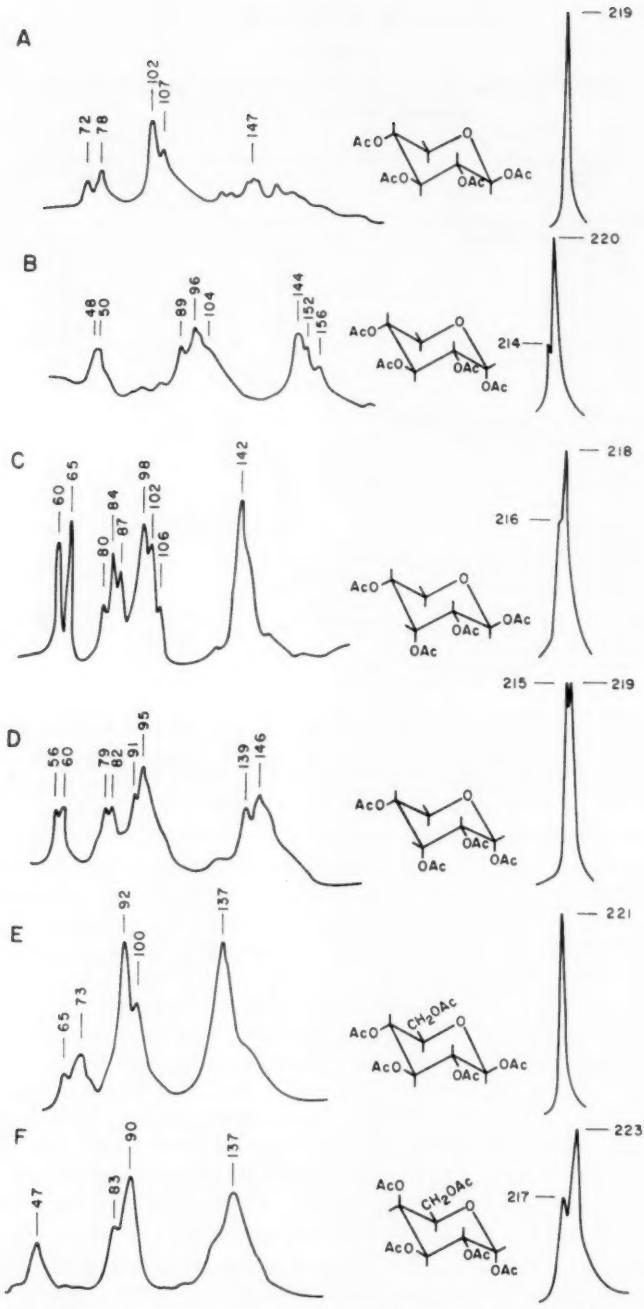
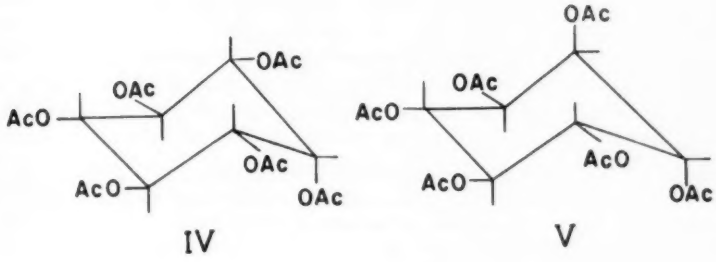


FIGURE 4.—Proton magnetic resonance spectra for some acetylated aldopyranoses: β-D-xylose tetraacetate (*A*); α-D-xylose tetraacetate (*B*); β-D-ribose tetraacetate (*C*); α-D-ribose tetraacetate (*D*); β-D-glucose pentaacetate (*E*); α-D-glucose pentaacetate (*F*).

It was observed that axial acetoxy groups tend to produce their signal at lower field than do equatorial acetoxy groups. Indication of this is seen in the spectra shown in Figure 4. Thus, β-D-xylose tetraacetate, which has only equatorial acetoxy groups, produced a single sharp peak for the acetoxy group hydrogens at 219 c.p.s. However, the spectrum for the α-anomer, which has the 1-acetoxy group in axial orientation, has the signal for the acetoxy groups in the form of a doublet. Although the signals are not completely resolved, there can be no doubt that the relative intensities are 1:3. The spectrum for β-D-ribose tetraacetate also indicates that one of the acetoxy groups produces its signal at lower field than do the other three acetoxy groups in the molecule. The spectrum of α-D-ribose tetraacetate, which possesses two axial and two equatorial acetoxy groups, has a symmetrical doublet for the hydrogens of the acetoxy groups. The assignment of the signals at low field in these doublets to the axial acetoxy groups was supported by the spectrum of α-D-glucose pentaacetate in which the hydrogens in the axial 1-acetoxy groups were replaced by deuterium. The signal at 217 c.p.s. (see Fig. 4) was reduced to a very low level. As expected from these results, the spectrum for myo-inositol hexaacetate (IV) has two signals with relative intensities 1:5 for the acetoxy group hydrogens and that for levo-inositol hexaacetate (V) possesses two signals for these hydrogens with relative intensities 1:2.



IV V

Our recent application (**10**) of the above described features of the NMR spectra of organic compounds which contain six-membered rings to the products which were obtained on the iodoacetoxylation of 3-methoxycyclohexene nicely illustrates the value of these findings to organic chemists. A similar application was previously made to establish the configurations of 1,3-dimethoxycyclohexanols (**11**). Treatment of 3-methoxycyclohexene with one mole of silver acetate and one mole of iodine is expected, as is shown in Figure 5, to yield four isomeric compounds. In view of the above described features of NMR spectra and empirically established chemical shifts for the various kinds of hydrogens which occur in organic molecules, it was possible to anticipate with confidence the NMR spectra for the isomeric 1,2-iodoacetoxy-3-methoxycyclohexanes. The theoretical spectra for

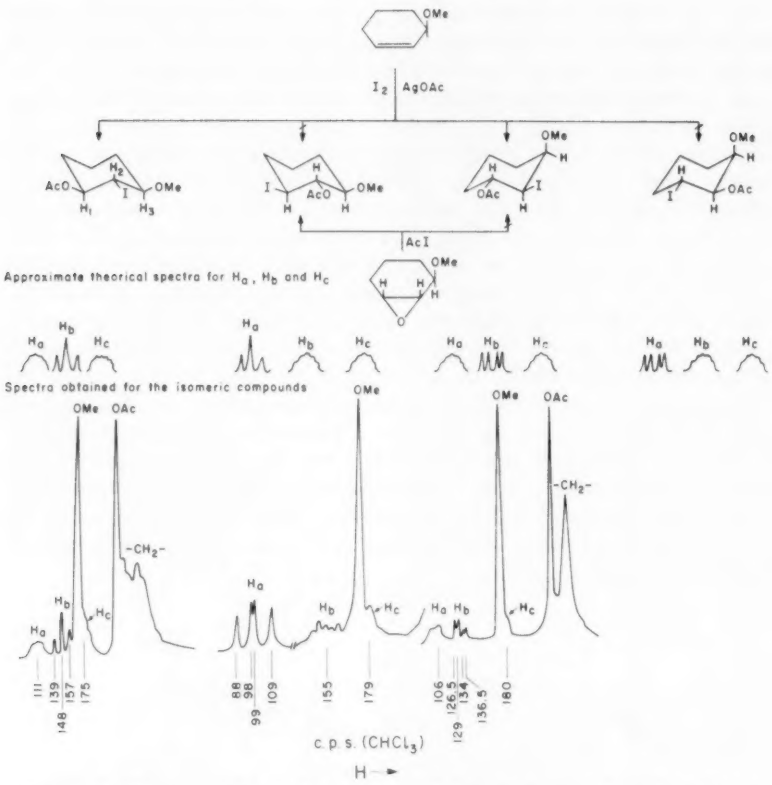


FIGURE 5.—The preparation and assignment of the structures and configurations to three isomeric 1,2-iodoacetoxy-3-methoxycyclohexanes on the basis of their proton magnetic resonance spectra. The signals in the spectra for the hydrogens on the carbons bonded to the acetoxy, iodine, and methoxy groups are labelled H_a, H_b and H_c, respectively. H_c is always at the 3-position.

the 1-,2-and 3-hydrogens are shown above those observed for the compounds in Figure 5. The theoretical spectra are based on a spin–spin coupling constant of 8 c.p.s. for two axial hydrogens on neighbouring carbons and one of 3 c.p.s. for hydrogens on neighbouring carbons one or both of which is equatorial. As already pointed out, the hydrogens of the methoxy and acetoxy groups produce single sharp peaks of intensity three. The 1- and 3-hydrogens must be expected to yield unresolved multiplets since, as illustrated in Figure 5, these hydrogens are coupled with the methylene groups at positions 6 and 4, respectively, as well as with the 2-hydrogen. On the other hand, the signal for the 2-hydrogen should be in the form of a quartet since, in each case, there is a large chemical shift on going from

the 2-hydrogen to either the 1- or 3-hydrogens as compared to the magnitudes of the coupling constants. However, it must be expected that the signal of an axial 2-hydrogen will have two of the bands of the quartet very close together and appear as a triplet with the intensity of the central band about double that of each side-band if the 1- and 3-hydrogens are also in axial orientation. This fact is illustrated by situation *e* in Figure 2. On the other hand, the magnitudes of the coupling constants are such that there should be no difficulty in obtaining a rather symmetrical quartet when an axial 2-hydrogen is coupled with an axial 1-hydrogen and an equatorial 2-hydrogen. This fact is illustrated by situation *c* in Figure 2.

The reaction of 3-methoxycyclohexene yielded two crystalline isomers VI and VII in 75 per cent and 20 per cent yields, respectively. A comparison of their spectra with the four theoretical spectra allowed a completely unequivocal conclusion as to their structures and configurations. A third isomer (VIII) was obtained by reaction of trans-3-methoxycyclohexene oxide with acetyl iodide. Again, as seen in Figure 5, the NMR spectrum of this compound provided convenient and unequivocal proof of the structure and configuration.

Similar applications of NMR spectroscopy to problems of structure have been made in other fields of organic chemistry (**1, 12**). For example, Rogers and Schoolery (**13**) have established correlations between the positions of the angular methyl groups of steroids and the configuration of the steroid nucleus. The technique is particularly useful for determining the points of substitution in benzene (**12**), pyridine (**14**), furan (**15**), and other such unsaturated ring structures since the fine structure of the residual hydrogens on the ring can provide information on the number of hydrogens on the neighbouring carbons (**1**). Obviously, an almost endless number of such applications of high-resolution nuclear magnetic resonance spectroscopy can be envisaged for gaining information on the immediate environment of atoms in organic molecules and this development is rapidly finding application in all fields of organic chemistry.

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